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First report of *Singerocybe* in Thailand

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Abstract — Specimens collected in Thailand have been identified as *Singerocybe alboinfundibuliformis*, based on morphology and ITS molecular analysis. A description and illustration are provided. This is the first record of the genus from Thailand.

Key words — *Agaricales*, *Tricholomataceae*, basidiomycete, gill fungus, taxonomy

Introduction

Harmaja (1988) erected *Singerocybe* (*Agaricales, Tricholomataceae*) with *S. viscida* Harmaja as the type species. This genus is distributed worldwide in temperate and tropical regions. *Singerocybe* species are saprotrophic and usually grow on soils rich in humus, dead wood, or rotting leaves (Peck 1873, Harmaja 1988, Takahashi 2000, Seok et al. 2009, Qin et al. 2014). Although no *Singerocybe* species has been reported from Thailand (Chandrasrikul et al. 2011), during a taxonomic survey of macrofungi collected in northern Thailand, we found specimens that corresponded to the description of *Singerocybe alboinfundibuliformis,* a species previously reported from China, Korea, and Japan (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). Here, we describe and illustrate the morphological characters of the Thai material and provide results from ITS sequence analysis.

Materials & methods

Morphology studies

Basidiocarps were collected from Doi Suthep-Pui National Park, Chiang Mai Province, Thailand in 2015 and wrapped in aluminum foil or kept in plastic specimen boxes for transport to the laboratory where notes on macromorphological features and photographs were taken within 24 h. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C; sections of dried material were mounted in 95% ethanol and rehydrated in distilled water, 3% KOH, or Melzer's reagent for microscopical examination; at least 50 measurements were made of each structure. The collections are deposited at the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU).

Molecular studies

Genomic DNA of three specimens was extracted from fresh tissue using CTAB according to Kumla et al. (2012). The internal transcribed spacer (ITS) region of ribosomal RNA gene was amplified by polymerase chain reaction (PCR) with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. Negative controls lacking fungal DNA were run to check for reagent contamination. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company (Selangor, Malaysia) using the ITS4 and ITS5 PCR primers. Sequences were used to query GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

Results

Taxonomic description

Singerocybe alboinfundibuliformis (Seok, Yang S. Kim, K.M. Park, W.G. Kim, K.H. Yoo & I.C. Park) Zhu L. Yang, J. Qin & Har. Takah., Mycologia 106: 1022 (2014). Plate 1

Pileus 20–50 mm in diameter, infundibuliform, hollow down to the base of stipe, smooth, white (4A1) to yellowish-white (4A2), margin involute when young and incurved in age. Lamellae decurrent, ≤1.5 mm wide, white (4A1) when young, becoming yellowish-white (4A2) to orange-white (5A2) in age. Stipe central, 20–45 × 3–10 mm, cylindrical or tapering towards the base, smooth, similar in color to the pileus. Odor strong. Pileipellis composed of irregularly parallel hyphae that are 2–5 µm wide, septate, and interspersed with vesicles (20–45 × 3–10 µm); vesicles also found at the stipe apex. Clamp connections present. Basidia 22.0–33.0 × 4.5–6.0 µm, clavate, 4-spored, with basal clamp connections and long sterigmata (≤5 µm). Basidiospores

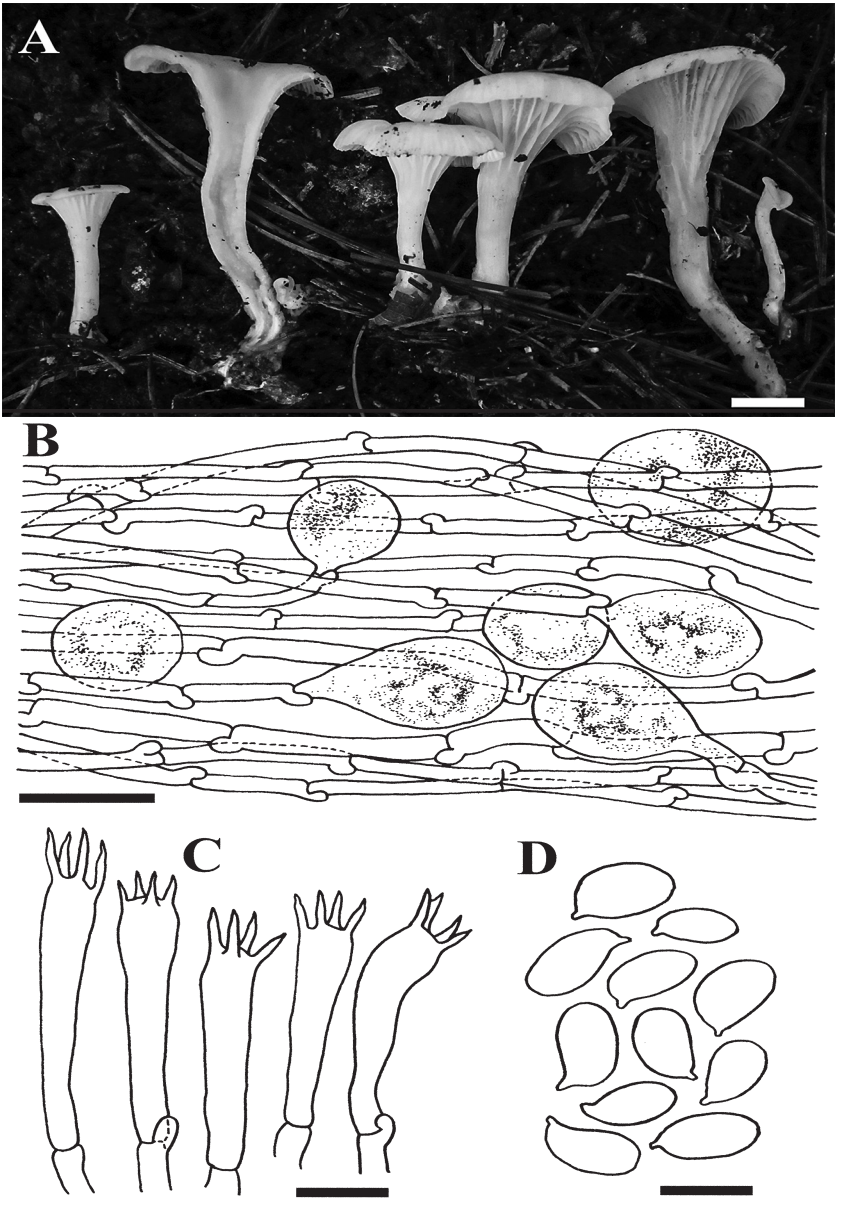

Plate 1. *Singerocybe alboinfundibuliformis* (SDBR-CMU-SGC72). A: basidiocarps; B: pileipellis; C: basidia; D: basidiospores. Scale bars: A = 10 mm, B = 25 µm, C = 10 µm, D = 5 µm.

3.0–6.5 × 2.0–4.0 µm (n = 50), Q = 1.2–2.2, elliptical, smooth, thin-walled, inamyloid. Spore print white.

Specimens examined — **THAILAND**, **Chiang Mai Province**, Muang District, Doi Suthep-Pui National Park, Yod Doi Pui, 18°49′16″N 98°53′35″E, elevation 1495 m, on rotting litter in mixed evergreen hill forest, 4 July 2015, Suwannarach N. (SDBR-CMU-SGC61; GenBank KT373850); 4 August 2015, Kumla J. & Suwannarach N. (SDBR-CMU-SGC85; GenBank KT373852); Doi Pui, 18°49′30″N 98°53′36″E, elevation 1515 m, on rich humus soil in mixed evergreen hill forest, 26 July 2015, Suwannarach N. (SDBR-CMU-SGC72; GenBank KT373851).

Molecular analysis

The ITS sequences of specimens SDBR-CMUSGC61 (702 bp), SDBR-CMUSGC72 (694 bp), and SDBR-CMUSGC85 (696 bp) were deposited in GenBank. All ITS sequences obtained in this study showed 99% similarity with *Singerocybe alboinfundibuliformis* JX514126 and JX514132.

Discussion

The presence of vesicles in the cuticle of the pileus and stipe support our collections in *Singerocybe*. All three specimens collected in northern Thailand were initially identified as *S. alboinfundibuliformis* based on descriptions by Takahashi (2000) and Seok et al. (2009) and the key provided by Qin et al. (2014). This species is easily distinguished from other *Singerocybe* species by a pileus that is deeply infundibuliform down to the base of the tubular stipe, the narrow and intervenose gills, and elliptical smooth basidiospores. *Singerocybe alboinfundibuliformis* resembles *S. humilis* (Berk. & Broome) Zhu L. Yang & J. Qin, and both are clearly distinguished from other species by their deeply infundibuliform pilei, open tubular stipes, and obviously intervenose gills (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). However, *S. humilis* is separated from *S. alboinfundibuliformis* by its very narrow gills with interstices becoming transversely reticulate to subporoid and DNA sequence analyses (Qin et al. 2014). *Singerocybe humilis* occurs in South Asia, while *S. alboinfundibuliformis* appears restricted to East Asia (Takahashi 2000, Seok et al. 2009, Qin et al. 2014). *Singerocybe hydrogramma* (Bull.) Harmaja, *S. phaeophthalma* (Pers.) Harmaja, and *S. viscida* are known only from Europe (Kuyper 1981, Harmaja 1988), *S. adirondackensis* (Peck) Zhu L. Yang & J. Qin from North America (Peck 1873; Qin 2014); *S. clitocyboides* (Cooke & Massee) Zhu L. Yang et al. from Australia and New Zealand (Pegler 1965), and *S. umbilicata* Zhu L. Yang & J. Qin from East Asia (Qin et al. 2014).

The molecular analysis also confirmed the three Thai specimens as *S. alboinfundibuliformis*. Therefore, the combination of morphological and molecular characters help establish *S. alboinfundibuliformis* as a new record in Thailand.

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